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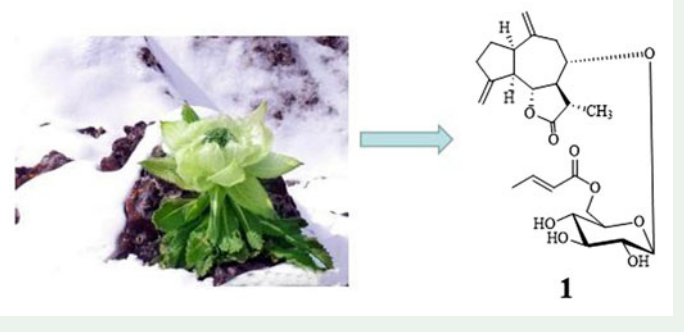
A new sesquiterpenoid glycoside from *Saussurea involucrata*

Shizhou Qi, Yiren Yang, Xiaoyan Xian, Xianzhe Li and Huiyuan Gao

School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, People's Republic of China

ABSTRACT

Saussurea involucrata, known for the abundant bioactive components, is a precious traditional Chinese medicine. In this study, a novel guaiane sesquiterpenoid glycoside named (1*R*, 5*R*, 6*R*, 7*R*, 8*S*, 11*S*)-11, 13-dihydrodehydrocostuslactone-8-*O*-6′-2′′(*E*)-butenoyl-*β*-D-glucopyranoside (**1**), together with seven known compounds (**2**–**8**) were isolated from the dried aerial part of *S. involucrata*. Their structures were elucidated by spectroscopic and physico-chemical analyses. The antioxidant and anti-inflammatory activities of compound **1** were investigated. And compound **1** showed weak radical scavenging activity and low inhibitory activity on nitric oxide (NO) production.



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antioxidant; anti-inflammatory; Saussurea involucrata; sesquiterpenoid glycoside

1. Introduction

Saussurea involucrata (Kar. et Kir.) Sch. -Bip (Asteraceae), named 'Tianshan Snow Lotus', 'Xinjiang Xuelian' or 'Xuelian Hua' in China, is a rare, slow growth and very precious Chinese medicinal herb (Flora of China Editorial Committee 1999). As a medicine which was recorded in Pharmacopoeia of People's Republic of China, *S. involucrata* was used to treat rheumatoid arthritis (Li et al. 1980), cough (Chik et al. 2015), dysmenorrhea (National Institutes for Food and Drug Control 1984), altitude sickness and

CONTACT Huiyuan Gao sypugaohy@163.com
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stomachache (Yi et al. 2010). Previous phytochemical and biological investigations revealed that Saussurea genus has numerous bioactive compounds such as coumarins (Jia et al. 1983), lignan, flavonoids (Xu et al. 2009), sesquiterpene lactones (Cao et al. 2016; Ren et al. 2007), steroids as well as phenylpropanoids (Seilgazy et al. 2017). Recent pharmacological studies demonstrated that some compounds of *S. involucrata* possessed a wide range of biological activities including anti-inflammatory, analgesic (Zhai et al. 2010), anti-fatigue (Jia and Wu 2008), anti-aging, anti-oxidative, anti-hypoxia, and hormonal-related gynecological disorders (Chik et al. 2015). In our previous *in vivo* study, the dried aerial parts of *S. involucrata* showed significant anti-arthritic effect (Han et al. 2016). And this result further stimulated us to explore the bioactive constituents of *S. involucrata*. Herein, we report the isolation and structural elucidation of a novel guaiane sesquiterpene glycoside, along with seven known compounds. New compound was evaluated for its antioxidant and anti-inflammatory activities. In addition, compound **3** was isolated from this genus for the first time, and compounds **7** and **8** were isolated from this plant for the first time.

2. Results and discussion

Compound **1**, obtained as colorless needles, gave a molecular formula of $C_{25}H_{34}O_9$ according to an $[M+Na]^+$ ion at *m/z* 501.2173 (calcd for $C_{25}H_{34}O_9Na$, 501.2101) in its HR-ESI-MS spectrum. The IR (KBr) spectrum showed absorptions attributable to hydroxyl (3427 cm^{-1}), carbonyl (1742 cm^{-1}) groups and olefinic bond (1654 cm^{-1}). The ^{1}H-NMR spectrum showed six olefinic protons at δ_H 6.88 (1H, m), 5.86 (1H, dd, $J=15.5$, 1.8 Hz), 5.02 (1H, br s), 4.95 (1H, br s), 4.86 (1H, br s) and 4.78 (1H, br s), two methyl groups at δ_H 1.82 (3H, d, $J=7.2$ Hz) and 1.28 (3H, d, $J=7.2$ Hz), two oxymethine groups at 3.91 (1H, t, $J=9.6$ Hz) and 3.65 (1H, td, $J=9.0$, 5.4 Hz), a sugar moiety at δ_H 4.10 (1H, dd, $J=11.4$, 7.8 Hz), 4.38 (1H, dd, $J=12.0$, 1.8 Hz), 4.35 (1H, d, $J=8.4$ Hz), 3.40 (1H, t, $J=8.4$ Hz), 3.17 (1H, t, $J=8.4$ Hz), 3.06 (1H, t, $J=9.0$ Hz) and 2.99 (1H, m), and three protons of hydroxyl groups in sugar unit at δ_H 5.21 (1H, d, $J=5.4$ Hz), 5.09 (1H, s), 5.05 (1H, d, $J=6.0$ Hz). And the *d* at δ_H 4.35 was assigned to an anomeric proton signal of the sugar moiety. The ^{13}C-NMR of **1** displayed twenty-five carbon signals. With the aid of HSQC spectrum, these carbon signals were classified as two ester carbonyl carbons at δ_C 178.8, 165.4, three pair of olefinic carbons at δ_C 145.3 and 113.6; 152.2 and 108.6; and 145.4 and 122.2, two oxymethine carbons at δ_C 83.2, 79.3, a sugar unit at δ_C 103.7, 76.8, 73.7, 73.6, 70.3, 63.5, and two methyl carbons at δ_C 17.7, 16.1. All these data indicated that compound **1** possessed the skeleton of sesquiterpene glycoside. And the structure could also be confirmed by comparing with NMR data of compound **2**. However, the 1 D and 2 D data of **1** were close to **2** except for the extra signals of an ester carbonyl signal (δ_C 165.4), a pair of olefinic signals (δ_H 6.88, δ_C 145.3; δ_H 5.86, δ_C 122.2) and a methyl signal (δ_H 1.82; δ_C 17.7). Furthermore, the downfield chemical shifts of C-5′ and C-6′ of **1** which appeared at δ_C 73.6 and 63.6 were also be observed. Combined with the key HMBC correlations of H-2″ (δ_H 5.86) to C-1″ (δ_C 165.4), C-4″ (δ_C 17.7), of H-6′ (δ_H 4.10, 4.38) to C-1″ (δ_C 165.4), and of H-4″ (δ_H 1.82) to C-2″ (δ_C 122.2), C-3″ (δ_C 145.3), the side chain which located at the C-6′ was established. The sugar moiety attached to C-8 was deduced by

the key HMBC correlation of H-1′ (δ_H 4.35) to C-8 (δ_C 83.2). The β-configuration of anomeric carbon of sugar moiety can be deduced on the basis of coupling constant of the anomeric proton ($J = 7.8$ Hz). And acid hydrolysis of **1** provided a D-glucose moiety using HPLC method (t_R: 18.5) (Tanaka et al. 2007).

Moreover, the NOESY correlations between H-1 (δ_H 2.88) and H-7 (δ_H 2.19); H-8 (δ_H 3.65), H-6 (δ_H 3.91) and H-11 (δ_H 2.67); and H-7 (δ_H 2.19), H-1 (δ_H 2.88), H-5 (δ_H 2.77) and H-9 (δ_H 2.18, 2.75), suggested that H-1, H-5 and H-7 exhibited α orientation, and H-6, H-8 and H-11 exhibited β orientation. The absolute configuration of **1a** could be gained by the comparison of the experimental and calculated electronic circular dichroism (ECD) spectra (Xiao et al. 2011). The experimental ECD spectrum of **1a** which possess the positive Cotton effect at 237 nm was consistent with the theoretical ECD spectrum of **1a**. Therefore, compound **1** was determined as (1*R*, 5*R*, 6*R*, 7*R*, 8*S*, 11*S*)-11, 13-dihydrodehydrocostuslactone-8-*O*-6′-2″(*E*)-butenoyl-β-D-glucopyranoside.

The known compounds were determined to be 11β,13-dihydrodehydrocostuslactone-8α-*O*-β-D-glucopyranoside (**2**) (Wang et al. 2007), flazine (**3**) (Su et al. 2002), involucratolactone (**4**) (Li and Jia 1989), 11α, 13-dihydrozaluzanin C (**5**) (Strapasson et al. 2012), dehydrocostuslactone (**6**) (Julianti et al. 2011), zaluzanin D (**7**) (Julianti et al. 2011), cinnamic acid (**8**) (Ferreira et al. 2005) by comparing their NMR data with literatures.

The new compound **1** was tested for its antioxidant avtivity through DPPH, ABTS free radical scavenging and FRAP assay. The anti-inflammatory activity of **1** was also evaluated in RAW 264.7 cells which stimulated by LPS. Compound **1** did not show significant radical scavenging activity or obvious inhibitory activity on nitric oxide (NO) production. This study provides a potentially contribution for the search of antioxidant and anti-inflammatory drugs.

3. Experimental

3.1. General experimental procedures

NMR spectra were obtained by Bruker AV-400 and AV-600 spectrometers (Bruker, Billerica, MA, USA) using tetramethyl silane as an internal standard. HR-ESI-MS spectra were obtained using a Bruker micro-TOF-Q mass spectrometer, IR spectra were obtained with a Bruker IFS-55 Fourier transform infrared (FT-IR) spectrometer (Bruker). CD spectrum was recorded with a Jasco CD-2095-plus circular dichroismdetector (JASCO Corporation, Tokyo, Japan). Open-column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd.), macroporous adsorption resin D101 (Langfang Nanda resin Co., Ltd.), and Sephadex LH-20 (Pharmacia Biotech, USA). TLC was performed with precoated silica gel GF_{254} glass plates (Qingdao Marine Chemical Co., Ltd). Preparative RP-HPLC was conducted on an Agela P1050 pump and Agela UV1000D UV spectrophotometric detector at 210 nm using a Mightysil RP-18 GP 250-20 column (5 μm, 20 $\times$ 250 mm).

3.2. Plant materials

The dried aerial parts of *S. involucrata* were purchased in February 2014 from Yuan Long Trade Co., Ltd. (Urumqi, Xinjiang, China), and identified by Associate Prof. Jincai

Lu of Shenyang Pharmaceutical University. A voucher specimen (ZB-14-XS012A) was deposited in the School of Traditional Chinese Medicine, Shenyang Pharmaceutical University, Shenyang, China.

3.3. Extraction and isolation

The dried aerial parts of *S. involucrata* (5.5 kg) were pulverized and extracted with $CHCl_3$ (44L × 2h × 3) to give 196.2 g crude extract. Then the *S. involucrata* was further extracted with 70% EtOH (44L × 2h × 3). The crude extract (2.4 kg) was suspended in H_2O and partitioned with EtOAc and *n*-BuOH to yield an EtOAc (128.6 g), *n*-BuOH (295.1 g) and aqueous (1.75 kg) fractions. The EtOAc part (120 g) was subjected to silica gel CC eluted with a gradient of CH_2Cl_2-CH_3OH (from 1: 0 to 0: 1) to obtain 10 fractions. Fraction 8 (2.6 g) was was chromatographed over a silica gel column using a gradient of CH_2Cl_2-MeOH (from 50: 1 to 0: 1), and was separated into 9 fractions (Fr.8.1–Fr.8.9). Furthermore, Fr.8.7 (102 mg) was purified by Spehadex LH-20 CC, and then separated by HPLC, using an gradient solvent system 40%-45% MeOH in H_2O over 60 min yielded compounds **1** (5.2 mg, t_R = 46.2 min), **2** (13.3 mg, t_R = 49.4 min). Fr.8.4 (800 mg) was subjected to the RP-18 column and eluted with MeOH-H_2O (3:10 to 10:0) to afford 8 fractions (Fr.8.4.1–Fr.8.4.8). Fr.8.4.4 (75.1 mg) was separated by HPLC, using an gradient solvent system 50%-70% MeOH in H_2O over 70 min yielded compounds **7** (8.2 mg, t_R = 35.1 min), **4** (10.9 mg, t_R = 54.7 min) and **5** (15.2 mg, t_R = 56.4 min). Fr.8.4.6 (118.7 mg) was purified via preparative HPLC using an isocratic solvent system of 65% MeOH in H_2O over 60 min yielded compounds **6** (8.1 mg, t_R = 41.2 min), **3** (10.9 mg, t_R = 48.1 min) and **8** (21.2 mg, t_R = 55.8 min).

Compound **1**: (1*R*, 5*R*, 6*R*, 7*R*, 8*S*, 11*S*)-11, 13-dihydrodehydrocostuslactone-8-*O*-6′-2″(*E*)-butenoyl-*β*-D-glucopyranoside, colorless needles; $[\alpha]_D^{20}$ + 82.9 (*c* 0.09, CH_3OH); IR (KBr) ν_{max} 3427, 2921, 1742, 1717, 1653, 1381, 1073, 974, 899 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 600 MHz) δ 6.88 (1H, m, H-3″), 5.86 (1H, dd, *J* = 15.5, 1.7 Hz, H-2″), 5.21 (1H, d, *J* = 5.4 Hz, OH), 5.09 (1H, s, OH), 5.05 (1H, d, *J* = 6.0 Hz, OH), 5.02, 4.95 (2H, br s, H-15), 4.86 (1H, br s, H-14), 4.78 (1H, br s, H-14), 4.36 (1H, m, H-6′), 4.30 (1H, d, *J* = 7.8 Hz, H-1′), 4.10 (1H, dd, *J* = 11.7, 7.5 Hz, H-6′), 3.91 (1H, t, *J* = 9.6 Hz, H-6), 3.65 (1H, m, *J* = 9.6, 5.2 Hz, H-8), 3.40 (1H, m, H-3′), 3.17 (1H, m, H-2′), 3.06 (1H, m, H-4′), 2.99 (1H, m, H-5′), 2.88 (1H, q, *J* = 7.8 Hz, H-1), 2.77 (1H, br t, *J* = 9.6 Hz, H-5), 2.75 (1H, dd, *J* = 13.2, 9.6 Hz, H-9), 2.67 (1H, dd, *J* = 10.2, 7.2 Hz, H-11), 2.44, 2.38 (2H, m, H-2), 2.19 (1H, q, *J* = 9.6 Hz, H-7), 2.18 (1H, dd, *J* = 13.2, 5.2 Hz, H-9), 1.82 (1H, dd, *J* = 7.2, 1.6 Hz, H-4″), 1.82, 1.74 (2H, m, H-3), 1.28 (3H, d, *J* = 7.2 Hz, H-13); ^{13}C-NMR (DMSO-d_6, 150 MHz) δ 178.8 (C-12), 165.4 (C-1″), 152.2 (C-4), 145.4 (C-10), 145.3 (C-3″), 122.2 (C-2″), 113.6 (C-14), 108.6 (C-15), 103.7 (C-1′), 83.3 (C-8), 79.3 (C-6), 76.8 (C-2′), 73.7 (C-5′), 73.6 (C-3′), 70.3 (C-4′), 63.5 (C-6′), 53.0 (C-7), 52.2 (C-5), 46.5 (C-1), 44.1 (C-9), 40.2 (C-11), 31.9 (C-2), 29.4 (C-3), 17.7 (C-4″), 16.1 (C-13); HR-ESI-MS: *m/z* 501.2173 $[M + Na]^+$ (calcd for $C_{25}H_{34}O_9$, 478.2203).

3.4. Acid hydrolysis of compound 1

Compound **1** (5 mg) dissolved in MeOH was mixed with 10% HCl (1.0 mL) and refluxed for 4 h. The cold hydrolysate was diluted 2-fold with H_2O, and extracted with EtOAc. 2

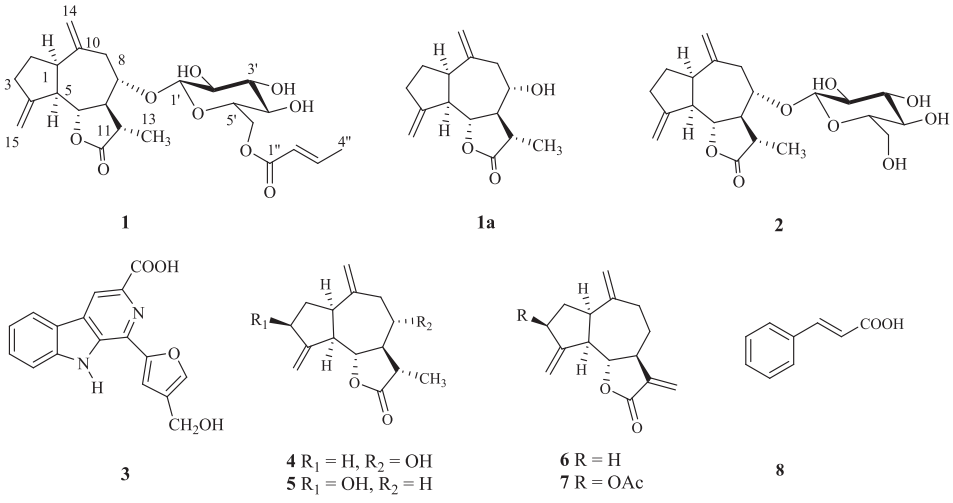

Figure 1. The structures of compounds **1**–**8**.

M ammonium hydroxide was used to neutralize the aqueous layer and a residue was obtained *in vacuo*. Then residue was analyzed by TLC over silica gel and by comparison with authentic samples. The residue was further analyzed by dissolving in pyridine (0.4 mL) which contain $_L$-cysteine methyl ester hydrochloride (2mg) and kept at 60 °C for 2 h. Next, *O*-Toylisothiocyanate (2 mL) was added and the mixture was heated at 60 °C for 1 h. The reaction mixture was analyzed by HPLC and a C_{18} HPLC column (4.6 × 250 mm, 10 mm particle size) was used [flow: 0.8 mL/min; mobile phase: CH_3CN-H_2O (25:75) containing 50 mM H_3PO_4]. Compared with the retention times of L-glucose (t_R = 18.7 min), L-glucose (t_R = 17.1 min), the structure of L-glucose of **1** was deduced by the retention times of 18.5 min.

3.5. Anti-inflammatory activity assay

NO production was determined by evaluating the level of NO_2^{2-} using Griess reaction. Briefly, RAW264.7 cells were plated in a 96 well plate at a density of 5×10^4 cells/well. After cells were treated with different concentrations of compound **1**, then were incubated and stimulated with LPS (100 ng/mL) for 24 h. And Griess reagent was added to simple to determine NO production. The absorbance was gained at 540 nm by a Varioskan flash instrument. NO production was calculated using the $NaNO_2$ standard curve and minocycline was used as a positive control.

3.6. Antioxidant activity assay

Antioxidant activity was evaluated by DPPH, ABTS free radical scavenging and FRAP assay. And trolox was used to be the positive control. DPPH, ABTS free radical scavenging assays were carried out according to the previous method (Peng et al. 2016). The free radical scavenging capability was calculated by the equation: RSA % = [(OD control - OD sample)/(OD control - OD blank)] × 100%. FRAP assay was determined by the published method (Wang et al. 2007). A calibration curve was established by

different concentrations of $FeSO_4$ at 0.15 to 5.00 mM and results were expressed as mmol of Fe^{2+} equivalents per gram.

4. Conclusion

Based on the result that the dried aerial parts of *S. involucrata* showed significant anti-arthritic effect in Wistar rats, eight bioactive compounds (**1–8**) including a novel sesquiterpene glycoside of were isolated from the dried aerial part of *S. involucrata*. And the structure of a 2-butenoic acid methyl ester group linked with sugar unit of **1** is rare. Except for the novelty of **1**, compound **3** was first isolated from the this genus and **7** and **8** were first isolated from *S. involucrata*. In addition, the antioxidant and anti-inflammatory activities of **1** were evaluated and **1** didn't show any significant activity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Cao K, Qian W, Xu Y, Zhou Z, Zhang Q, Zhang XF. 2016. A new sesquiterpenoid from Saussurea lappa roots. Nat Prod Res. 30:2160–2163.

Chik WI, Zhu L, Fan LL, Yi T, Zhu GY, Gou XJ, Tang YN, Xu J, Yeung WP, Zhao ZZ, Yu ZL, Chen HB. 2015. *Saussurea involucrata*: A review of the botany, phytochemistry and ethnopharmacology of a rare traditional herbal medicine. J Ethnopharmacol. 172:44–60.

Ferreira AA, Azevedo AO, Silveira D. 2005. Constituents of *Lychnophora pinaster* hydroalcoholic extract. Chem Nat Compd. 41:466–466.

Flora of China Editorial Committee. 1999. Flora of China. Beijing, China: Science Press; 78:35–37.

Han XL, Su D, Xian XY, Zhou MY, Li XZ, Huang J, Wang JH, Gao HY. 2016. Inhibitory effects of *Saussurea involucrata* (Kar. et Kir.) Sch. -Bip. on adjuvant arthritis in rats. J Ethnopharmacol. 194:228–235.

Jia JM, Wu CF. 2008. Antifatigue activity of tissue culture extracts of *Saussurea involucrata*. Pharm Biol. 46:433–436.

Jia ZJ, Li Y, Du M. 1983. Studies on the chemical constituents of *Saussurea involucrata* Kar. et Kin. (I). Gaodeng Xuexiao Huaxue Xuebao. 4:581–584.

Julianti T, Hata Y, Zimmermann S, Kaiser M, Hamburger M, Adams M. 2011. Antitrypanosomal sesquiterpene lactones from *Saussurea costus*. Fitoterapia. 82:955–959.

Li Y, Jia ZJ. 1989. Guaianolides form *Saussurea involucrate*. Phytochemistry. 28:3395–3397.

Li GH, Liu F, Zhao RC. 1980. Studies on pharmacological actions of *Saussurea involucrata* Kar et Kir ex Maxim. Acta Pharm Sin. 15:368–370.

National Institutes for Food and Drug Control. 1984. Zhongguo Minzu Yaozhi. Beijing: People's Medical Publishing House; 448–449.

Peng Y, Lou LL, Liu SF, Zhou L, Huang XX, Song SJ. 2016. Antioxidant and anti- inflammatory neolignans from the seeds of hawthorn. Bioorg Med Chem Lett. 26:5501–5506.

Ren G, Yu ZM, Chen YL, Wu SH, Fu CX. 2007. Sesquiterpene lactones from Saussurea alata. Nat Prod Res. 21:221–226.

Seilgazy M, Li J, Aisa HA. 2017. Isolation of steroidal esters from seeds of *Saussurea involucrata*. Chem Nat Compd. 53:1196–1198.

Strapasson RLB, Cervi AC, Carvalho JE, Salvador MJ, Stefanello MEA. 2012. Bioactivity-guided isolation of cytotoxic sesquiterpene lactones of gochnatia polymorpha ssp. floccosa. Phytother Res. 26:1053–1056.

Su BN, Chang LC, Park EJ, Cuendet M, Santarsiero BD, Mesecar AD, Mehta RG, Fong HH, Pezzuto JM, Kinghorn AD. 2002. Bioactive constituents of the seeds of *Brucea javanica*. Planta Med. 68: 730–733.

Tanaka T, Nakashima T, Ueda T, Tomii K, Kouno I. 2007. Facile discrimination of aldose enantiomers by reversed-phase HPLC. Chem Pharm Bull. 55:899–901.

Wang XL, Gesang SL, Jiao W, Liao X, Ding LS. 2007. Two new sesquiterpenoid glucosides from the aerial parts of *Saussurea involucrate*. J Integr Plant Biol. 49:609–14.

Xiao W, Li X, Li N, Bolati M, Wang XJ, Jia XG, Zhao, YQ. 2011. Sesquiterpene lactones from *Saussurea involucrata*. Fitoterapia. 82:983–987.

Xu YJ, Zhao DX, Fu CX, Cheng LQ, Wang NF, Han LJ, Ma FS. 2009. Determination of flavonoid compounds from *Saussurea involucrata* by liquid chromatography electrospray ionisation mass spectrometry. Nat Prod Res. 23:1689–1698.

Yi, T, Zhao ZZ, Yu ZL, Chen HB. 2010. Comparison of the anti-inflammatory and anti-nociceptive effects of three medicinal plants known as Snow Lotus herb in traditional Uighur and Tibetan medicines. J Ethnopharmacol. 128:405–411.

Zhai KF, Duan H, Xing JG, Huang H. 2010. Study on the anti-inflammatory and analgesic effects of various purified parts from *Saussurea involucrata*. Chin J Hosp Pharm. 5:374–377.